Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

HYDROGEN-ION STUDIES

II. CHANGES IN THE REACTION OF SERUM ON THERMAL DESTRUCTION OF COMPLEMENT *

Edwin F. Hirsch and Ebba C. Peters

From the Pathological Laboratory of St. Luke's Hospital, Chicago

Numerous attempts have been made to determine the chemical nature of the thermolabile substance "complement" in normal blood serum. Its resemblance in certain respects to an enzyme has suggested that it is a lipase or a protease. Other properties suggest that it is a protein or lipin, or possibly a combination of soaps or lipins with serum proteins. Complement in serum is said to consist of two portions, an "end-piece" present in the albumin fraction and a "mid-piece" contained in the globulin portion. Bronfenbrenner and Noguchi[1] regard this so-called complement-splitting phenomenon merely an inactivation of the complement and not an actual cleavage. Brooks[2] recently expressed the opinion that the hemolytic action (complement) of serum is due primarily to a single substance which may be destroyed by ultraviolet rays according to a monomolecular reaction. There is considerable evidence, he says, that fatty acids are important in immune reactions and this taken with other facts known for complement suggests the presence of some fatty-acid compound acting as hemolysin in complement. He proposes that serum contains a hemolytic substance derived from a precursor (may resemble lecithin), and is constantly being formed and simultaneously being broken down.

Most of the methods for investigating complement have subjected normal serum to the action of chemicals. These modify to a great extent the menstruum in which complement exists and may even change the substance or substances constituting the complement. The extreme thermolability of complement makes a direct chemical examination very difficult. It seems possible with the precision of the gas-chain method to study the changes in the H-ion concentration of serum on heating to 56 C., and perhaps in this way to gain new information regarding the chemical behavior of complement.

Received for publication Oct. 21, 1921.

* Aided by the Winfield Peck Memorial Fund.

[1] Jour. Exper. Med., 1912, 15, pp. 598 and 625.

[2] Jour. Gen. Physiol., 1920-21, 3, p. 185.

Normal guinea-pig and rabbit serum was used in these experiments. The serum separating from coagulated blood kept in the icebox over night was thoroughly mixed in a large clean glass tube, and then distributed quickly into 4 c c glass test tubes, each one filled to within a short distance of the top and closed with a paraffined stopper. The tubes were heated in a water bath at 56 C. for periods of 1 minute to 2 hours, the length of time being measured from the moment the tube was placed in the water bath until it was removed. This method, of course, in the short intervals disregards the lowering of the temperature of the water bath caused by introducing the tubes, as well as the time necessary to bring the serum and water in the bath to 56 C. On the other hand, the tendency by this method is to extend over longer periods of time any changes in the H-ion concentration brought about by heating the serum. Immediately after heating, the tubes were chilled in an ice bath, and kept there until the H-ion

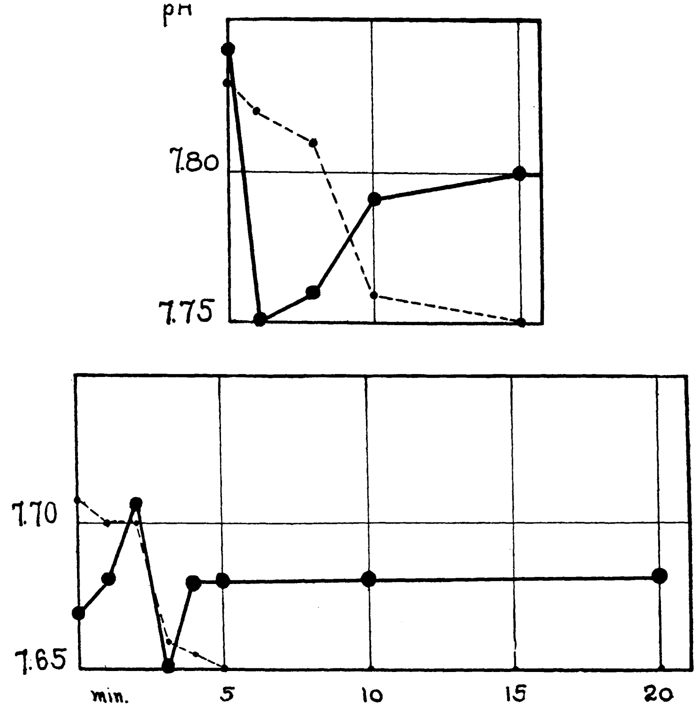

Chart 1.—Changes in the reaction of guinea-pig serum during thermal destruction of the complement. The continuous line represents the hydrogen-ion concentration; the broken line represents hemolysis.

determinations were made. About 2 hours were required to complete a single experiment (9 H-ion determinations). Sealing the tubes and chilling them immediately after heating seemed to guard against reaction changes due to the escape of carbon dioxide, and those caused by autolysis; at least, such changes were reduced to a minimum. The H-ion concentration of unheated serum was determined with separate portions at the beginning and at the end of each experiment. These estimations were made with the $\text{Hg-HgCl-}\frac{N}{10}\text{-KCl} \mid \text{sat. KCl} \mid \text{PtH}_2$ system, a McClendon electrode being used to contain the serum. The electromotive force readings were made with a Leeds and Northrup type K potentiometer. This system was checked against known standard solutions, and all the estimations were made at room temperature (about 25 C.), there being little change in temperature during the period of each experiment. All the glass containers were washed with distilled water and dried, the McClendon electrode with water triple distilled in glass. The potentiometer readings were converted into the equivalent P_H expressions by the formula:

$$\frac{\text{E. M. F. (observed)} - \text{E (calomel electrode)}}{0.000\,1983\ \text{T}} = \log. \frac{1}{(\text{H}+)} = P_H.$$

The complement activity of the serum was determined by the usual sheep red corpuscle, rabbit amboceptor system, the guinea-pig serum being diluted to 10%, the rabbit to 40%.

The results of these experiments are contained in the table, and those of two are graphically represented in the chart. On heating normal guinea-pig or rabbit serum, there is a prompt change in its reaction. In the experiments made, there seems to be a H-ion concentration of about P_H 7.74 to 7.76 which determines the direction of the initial change in reaction. When the P_H is greater, namely, 7.77, etc., the serum becomes less alkaline; when the P_H is smaller, namely, 7.73, etc., it becomes more alkaline. This initial change frequently reaches its maximum during the first minute of heating. When the initial change in reaction is an increased alkalinity there usually follows within 3 to 5 minutes a return to approximately the original reaction, and then again a change to a more alkaline reaction which remains fairly constant after the complement has been completely destroyed. When the initial change in reaction is a diminished alkalinity there is a subsequent return but not as great an alkalinity as that of the original unheated serum.

The reaction of the unheated serum kept in the ice water bath during the period of experiment (2 hours) changed in each experiment in the same direction and practically to the same degree as the initial change caused by heating the serum. A slight variation from this was observed once with guinea-pig serum (8) and once with rabbit serum (9).

TABLE 1

TABLE OF RESULTS OBTAINED WITH HEATING GUINEA-PIG AND RABBIT SERUM TO 56 C.

No.	Kind	Serum P_H.											
		Heated 56 C. (Min.)										Unheated	
		1	2	3	4	5	10	20	30	60	120	0	120
1	Guinea-pig	7.75	....	7.76	....	7.79	7.80*	....	....	7.80	7.80	7.84	7.76
2	Guinea-pig	7.68	....	7.68	*	7.68	7.70	....	7.70	....	....	7.80	7.68
3	Guinea-pig	7.80	....	7.83	....	7.83	7.83*	....	7.82	7.82	7.82	7.85	7.80
4	Guinea-pig	7.79	....	7.80	....	7.75	7.79	....	7.76*	7.75	7.76	7.74	7.78
5	Guinea-pig	7.69	....	7.66	....	7.68	7.69	....	7.73*	....	7.72	7.76	7.68
6	Guinea-pig	7.75	7.74	7.78	7.76	7.75	7.75	7.76*	....	....	....	7.76	7.76
7	Guinea-pig	7.68	7.71	7.65	7.68	7.68*	....	7.68	....	....	....	7.67	7.71
8	Guinea-pig	7.47	....	7.49	7.49	7.51	7.47	7.51*	....	....	....	7.47	7.47
9	Rabbit	7.50	7.41	7.42	7.41*	7.41	7.41	7.41	....	....	....	7.40	7.42
10	Rabbit	7.46	7.49*	7.49	7.49	7.49	7.49	7.48	....	....	....	7.45	7.49
11	Rabbit	7.40	7.42	7.40	7.40	7.40†	....	7.40	....	....	....	7.38	7.43

* Complement destroyed completely.
† Slight hemolysis, serum not tested further.

DISCUSSION

An interpretation of the changes in the reaction of rabbit and guinea-pig serum when heated to 56 C. probably must be made on the basis of dissociation changes of a certain substance or substances (complement) in the serum. It seems that the dissociation of the complement substance occurs with the liberation of an acid radical, which spontaneously and with heating is volatilized or is removed by combining with some other substance. The H-ion concentration of serum seems to be the factor determining how much of this acid radical is present as such, above the P_H 7.74 very little, below this point more, so that when the serum is heated, in the former instance the complement substance rapidly dissociates, liberating its acid radical which then is removed by volatilization or chemical combination. In the latter instance its removal occurs first, and then dissociation and removal until the complement substance is completely destroyed.

The possibility that some volatile acid itself may be the complement suggested attempting to collect in inactivated serum (4 c c) the gases driven off from a larger amount (20 c c) of guinea-pig or rabbit serum during the heating at 56 C. for half an hour. These experiments demonstrated that such reactivation does not occur, and in order to be certain that a volatile acid substance was driven over, the H-ion concentration of inactivated serum was determined with a portion before, and with a portion after the gas had been forced through it. The reaction of the inactivated serum was changed by the substances volatilized (20 c c guinea-pig serum) from P_H 7.86 to 7.82, and from 7.81 to 7.76. That this change in reaction is not due entirely to carbon dioxide was demonstrated by testing inactivated serum before and after it had been exposed to pure carbon dioxide gas in amounts equal to the volume of gas driven off from heated serum. The reaction of each portion differed only to a slight degree. In order to satisfy the criticism that the glassware used contributed to the reaction changes, a set of tubes was thoroughly steamed under pressure in an auto clave, then placed in acid cleaning solution, thoroughly washed with water, finally with distilled water, and then dried. The results obtained with these tubes are similar to those obtained in the other experiments.

CONCLUSIONS

The destruction by heat (56 C.) of complement in normal guinea-pig and rabbit serum is accompanied by changes in the H-ion concentration. These probably are caused by dissociation of the complement substance or substances.